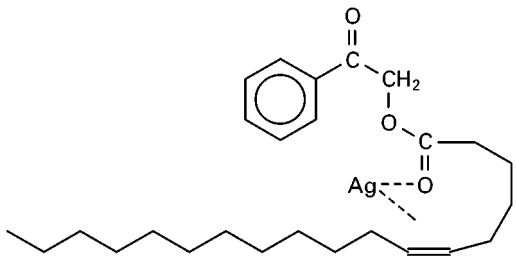

Figure 5 Schematic representation of the interaction of a silver ion with the phenacyl ester derivative of petroselinic acid.

esters. Experiments with esters with a variety of different electron-donating and electron-withdrawing substituents now provide firm evidence for this hypothesis.

An interaction between one silver ion and two double bonds at the same time may explain the chromatographic behaviour of fatty derivatives with two or more double bonds in the acyl chain in silver ion HPLC. When the distance between the double bonds is optimum, i.e. with a 1,5-*cis*,*cis*-diene system, fatty acids are very strongly retained, and the effect diminishes as the number of methylene groups between the double bonds is varied. If the double bonds interacted singly with silver ions, it might have been anticipated that the kinetics of the system would be such that retention would be comparable in magnitude to the sum of the individual parts, but this is clearly not so. This theory of complexation between silver ions and bis-double bond systems could potentially be applied to polyenoic fatty acid derivatives. It would predict that a triene would be held twice as strongly as a diene, a tetraene three times as strongly and so forth. Such a simple relationship is not found in practice (the degree of complex formation is even greater than anticipated), possibly because interactions with the ester moiety have to be taken into consideration and because the conformations of polyenes may permit some interactions between silver ions and double bonds that are remote from each other, via the formation of pseudo-cyclic structures.

Analogous physicochemical studies of the behaviour of triacylglycerols on silver ion chromatography suggests that a dual interaction is important in this instance also. For example, it has been shown that highly unsaturated triacylglycerols are retained especially strongly; a species with nine double bonds is held 10 000 times as strongly as one with a single double bond. It is the strength of this interaction rather than the efficiency of the column *per se* that is responsible for the quality of the separations. However, here further work is certainly necessary to confirm the mechanism.

See also: **III/Lipids:** Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Oils, Fats and Waxes: Supercritical Fluid Chromatography. Silver Ion:** Thin-Layer (Planar) Chromatography.

Further Reading

Christie WW (1987) A stable silver-loaded column for the separation of lipids by high-performance liquid chromatography. *Journal of High Resolution Chromatography*; *Chromatography Communications* 10: 148.

Christie WW (1988) Separation of molecular species of triacylglycerols by high-performance liquid chromatography with a silver ion column. *Journal of Chromatography* 454: 273.

Christie WW (1989) *Gas Chromatography and Lipids. A Practical Guide*. Dundee: The Oily Press.

DeJarlais WJ, Adlof RO and Emken EA (1983) Acetonitrile as eluent in silver resin chromatography. *Journal of the American Oil Chemists' Society* 60: 975.

Dobson G, Christie WW and Nikolova-Damyanova B (1995) Silver ion chromatography of lipids and fatty acids. *Journal of Chromatography B* 671: 197.

Laakso P and Christie WW (1991) Combination of silver ion and reversed-phase high-performance liquid chromatography in the fractionation of herring oil triacylglycerols. *Journal of the American Oil Chemists' Society* 68: 213–223.

Morris LJ (1966) Separation of lipids by silver ion chromatography. *Journal of Lipid Research* 7: 717.

Nikolova-Damyanova B (1992) Silver ion chromatography and lipids. In: Christie WW (ed.) *Advances in Lipid Methodology – One*, pp. 181–237. Dundee: The Oily Press.

Nikolova-Damyanova B, Christie WW and Herslöf BG (1995) Retention properties of triacylglycerols on silver ion high-performance liquid chromatography. *Journal of Chromatography A* 694: 375.

Nikolova-Damyanova B, Christie WW and Herslöf BG (1996) Mechanistic aspects of fatty acid retention in silver ion chromatography. *Journal of Chromatography A* 749: 47.

DropBooks

Thin-Layer (Planar) Chromatography

B. Nikolova-Damyanova, Bulgarian Academy of Sciences, Sofia, Bulgaria

Copyright © 2000 Academic Press

Introduction

Silver ions (Ag^+), like the ions of many other transition metals, interact specifically with

Table 1 List of compounds subjected to Ag-TLC

Class	*Compounds*
Lipids	Fatty acid mixtures
	Isomeric octadecenoic and octadecadienoic fatty acids
	Furanoid fatty acids
	Cyclopropene fatty acids
	Oxygenated unsaturated acids
	Triacylglycerol mixtures of natural fats and oils of terrestrial and marine origin
	Phospholipids
	Sterol esters
Terpenes	C_{10}, C_{15}, C_{20} terpenes
	Terpene alcohols
	Monoterpenes
Hydrocarbons	Acyclic olefines
	Alkylbenzenes
	Alkylphenylsulfides
Miscellaneous	Steroids
	Sterols
	Sterol acetates
	Derivatized unsaturated aldehydes and ketones
	Prostaglandins
	Hydroxyprogesterones
	Estrogens
	Mineral oils

unsaturated compounds to form complexes with olefinic double bonds. In 1938 Winstein and Lukas and in 1952 Nichols demonstrated that the interaction between Ag^+ and double bonds might be of interest for chemical analysis. By applying a liquid–liquid distribution system with Ag^+ present in the aqueous phase it was possible to separate easily unsaturated from saturated compounds and *E*- from *Z*-monounsaturated olefins. The great potential of this interaction for separation of unsaturated compounds was fully recognized when gas–liquid chromatography (GLC) and thin-layer chromatography (TLC) developed into routine analytical techniques.

The chromatographic technique that utilizes the interaction between Ag^+ and an olefinic bond to conduct the separation process is now called argentation (silver ion) chromatography. Argentation chromatography was first developed as a GLC technique. However, argentation TLC (Ag-TLC) soon became a basic separation method for the analysis of different types of unsaturated compounds. For many years it has been a most valuable method in lipid analysis, providing essential information about the lipid structure and composition.

Compounds that have been most frequently examined by Ag-TLC are listed in **Table 1**.

Silver Ion Complexation with Double Bonds

The model now considered to represent correctly the bonding between Ag^+ and a double bond was suggested by Dewar in 1951. It supposes the formation of a σ-type bond between the occupied $2p\pi$ orbitals of an olefinic double bond and the free 5s and 5p orbitals of the Ag^+, and a (probably weaker) π-acceptor backbond between the occupied 4d orbitals of the silver ion and the free antibonding $2p\pi^*$ orbitals of the olefinic bond (**Figure 1**).

It is suggested that a silver ion interacts with one mono-olefin molecule to give a planar complex with a triangular structure. However, there is evidence that a silver ion may interact with two ethylenic molecules. X-ray studies of crystalline silver ion complexes with some short chain aliphatic diolefins show that the Ag^+ is coordinated with two double bonds from different olefinic molecules.

The stability of the silver ion–double bond complex is influenced by the spatial arrangement of the overlapping orbitals, the basicity of and electronic effects in the olefinic molecule, and by solvent effects. Quantitative data (for example equilibrium constants) exist only for a number of short chain monoolefins, diolefins with accumulated, conjugated and separated methylene-interrupted double bonds, and for some cyclopenta- and cyclooctadienes. Most of these data, as well as the estimation of the relative strength of other complexes, are based on chromatographic measurements.

The general conclusions about complex formation reached so far are as follows:

1. Unsaturated acyclic and carbocyclic compounds form more stable complexes than do aromatics.
2. Carbocyclic compounds with a single exocyclic double bond form stronger complexes than do carbocyclic compounds with a single internal double bond. Cyclopenta- and cyclooctadienes

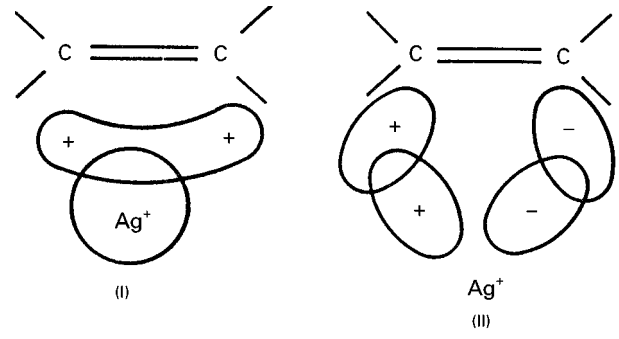

Figure 1 Schematic presentation of the interaction between a silver ion and a double bond.

form very stable complexes, especially when having a 1,5-diene system.

3. For acyclic compounds it has been found that:
   – the stability decreases with the increasing chain length;
   – the stability decreases with an increasing number of substituents at the double bond in the order $RCH{=}CH_2 > R_2C{=}CH_2 > R_2C{=}CHR > R_2C{=}CR_2$;
   – the stability increases when a hydrogen atom from a molecule of the RCH=CHR type is replaced with deuterium or tritium atom. The effect has been ascribed to greater electron release from a C–D than from a C–H bond, i.e. to the higher basicity of the deuterated molecule;
   – *cis* (*Z*)-isomers form stronger complex than do *trans* (*E*)-isomers. The greater stability of the *cis*-isomer is ascribed either to the relief of strain when the complex is formed or, more probably, to the steric hindrance of the double bond in *trans*-isomers;
   – diolefins form more stable complexes than do mono-olefins, but conjugated dienes form less stable complexes than do those with methylene-interrupted double bonds;
   – the stability increases with increasing distance between the double bonds. The most stable diene complexes are formed by the 1,5-diene system, perhaps because a chelate complex can be formed in the latter case.

Generally, the rate of complex formation for most acyclic compounds is very rapid. The complexes are usually unstable and exist in equilibrium with the free form of the olefin. The coordination forces seem to be very weak. The IR spectrum, for example, shows very little shift in frequencies from those of free double bonds. These particular properties of complexation between a double bond and a silver ion are favourable for use in chromatography.

The Technique of Ag-TLC

The Layer

Argentation TLC is utilized in two modifications: analytical and preparative. Both home-made and precoated plates (glass only) are used. Plate dimensions may vary from 5 cm × 20 cm to 20 cm × 20 cm. High performance TLC plates (HPTLC) have recently been found to be useful. Home-made layers, despite their messy preparation, are more versatile and often provide better separations than commercially prepared ones. Common adsorbents are listed in **Table 2**.

The thickness of the adsorbent layer ranges from 0.2 to 0.3 mm for analytical plates and from 0.5 to 1.0 mm for preparative plates. Fully automated spreaders for home-made layers are commercially available but simple spreaders are equally effective. However, some practice is needed for layer preparation and precoated plates are now mostly preferred.

The Incorporation of Silver Ions

There are two general ways to perform silver ion TLC of which by far the most common is to use a layer of adsorbent impregnated with a silver salt. It is possible as an alternative to add a silver salt to the mobile phase when a reversed-phase TLC separation is performed. This approach has found limited use only. Although the influence of the salt anion has not been studied systematically, there is evidence that its nature may affect the resolution. A list of silver salts used and their supposed effect is shown in **Table 3**.

Impregnation can be performed by incorporating the silver salt into the slurry of adsorbent used to make the layer. Also, the prepared plate can be immersed in a methanol, acetone or acetonitrile solution of the silver salt or sprayed with one of these solutions. Only the first approach affords proper control of the silver content of the layer. However, it is inconvenient and messy and is now rarely used. Since

DropBooks

Table 2 Adsorbents, frequently used with Ag-TLC

Adsorbent	*Compounds to be separated*	*Comments*
Silica gel G (binder is calcium sulfate)	Fatty acids	Fatty acids should be first converted into methyl esters.
	Triacylglycerols	Components are separated as intact compounds.
	Diacylglycerols	Compounds are separated after conversion into acetates.
	Polar lipids	Polar lipids should be converted into less polar derivatives, either by removing or by derivatizing the polar head.
	Terpenes	Compounds are usually separated as intact compounds.
Silica gel H (no binder)	Polar lipids	It is possible to separate polar lipids as intact compounds on this layer.
Alumina	Fatty acids	Species are separated as free fatty acids and as methyl esters. Alumina as adsorbent should be used with care since it is known to react with some analytes and solvents.

Table 3 List of silver salts used in Ag-TLC

Salts	*Components to be separated*	*Comments*
Silver nitrate	Fatty acids derivatives	The most frequently used silver salt with very broad application for various separations.
	Triacylglycerols	
	Polar lipids	
	Terpenes	
Ammonia solution of silver nitrate	Fatty acid methyl esters	Silver salts other than silver nitrate are supposed to improve the separation.
Silver sulfamate	Fatty acid methyl esters	
Silver benzenesulfonate	Fatty acid methyl esters	
Silver iodate	Terpenes	
Silver perchlorate	Terpenes	

it appears that the content of silver ions in the layer may not, in fact, be critical, immersion procedures are mostly used. They can be standardized sufficiently well to provide repeatable results. Spraying procedures are less easily controlled. Spraying may have to be repeated from two to six times until the adsorbent layer is properly wetted. Sufficient spraying of the layer is somewhat arbitrary and depends on personal skill and on the samples to be examined. Immersion or spraying are only applicable to precoated plates. These plates should be immersed in a silver salt solution (methanol or acetonitrile) for not less than 15–20 min. A dynamic impregnation technique has also been proposed. The plate is developed with a 10–20% solution of silver nitrate in acetonitrile. It has been claimed that in this way a gradient of silver ions is formed in the development direction. The gradient is claimed to improve the separation of triacylglycerols. The approach has not found wide application and its advantages cannot be estimated.

The silver content of the adsorbent layer varies between rather broad limits and differs for analytical and preparative plates (**Table 4**). Layers containing a high percentage of silver nitrate were considered necessary to achieve good analytical resolution. This high percentage is, however, very inconvenient. The plates are very sensitive to light and this can greatly hamper detection and quantification. Impregnation by immersion in 0.5% methanolic silver nitrate provides excellent results in the resolution of lipids, for example, without this disadvantage.

Table 4 Concentration of the silver nitrate solutions and methods for impregnation of a TLC layer

$AgNO_3$ (%)		*Impregnation*
Analytical plates	*Preparative plates*	
0.5–2	1–5	Immersion
5–30	5–20	Incorporation
10–40	40	Spraying

Treatment of the Plates and Precautions

Plates should be used immediately after being dried in air (for 1 h). Many workers activate the plates before use by heating at 110°C for about 1 h. However, good results have been reported after activation for only 5 min. Thus, it seems that the necessity for activation is questionable, and the analyst must trust to his or her own experience and the nature of the samples that are being handled. Activation has been found to be very important for silica gel H plates and temperatures higher than 110°C for periods of much longer than 1 h have been recommended.

Atmospheric humidity has an appreciable effect on separation, especially of highly unsaturated species. It is recommended that activated TLC plates be kept in a desiccator over drying agents (ideally in the dark). However, it is not easy to control humidity in practice. This may be one of the reasons for the relatively poor overall reproducibility of migration and resolution in separations performed by Ag-TLC.

Sample Preparation

Terpenes and triacylglycerols are applied as solutions of appropriate concentration in suitable solvents. Preliminary fractionation and purification from accompanying compounds is required. Fatty acids should be converted into less polar derivatives, usually into methyl esters. Recently, aromatic derivatives of fatty acids have proved to provide much better separation of difficult-to-resolve mixtures. Ag-TLC has not been very successful in separating intact complex lipids. Most of the reported procedures employ preliminary fractionation into specified classes and conversion of the latter into less polar derivatives.

Sample size is an important factor since overload greatly worsens the resolution. Analytical Ag-TLC on 0.2 mm thick layers requires samples of a maximum

of 30 μg. For preparative separation (0.5–1.0 mm layer thickness) the sample size can be scaled up to 80–100 mg, depending on the sample composition, the quantitative ratio between components and the required resolution.

Development

Most separation protocols recommend ascending development in covered tanks in which the atmosphere has been saturated with the vapour of the mobile phase. The saturation is considered to shorten the duration of development and often to improve the reproducibility. There are no firm data to support this conclusion and it might depend on the nature of the analyte. Poor separation and tailing of zones have also been reported under these conditions. Excellent separations of fatty acids and triacylglycerols are obtained without saturation of the atmosphere, or even in an open container. The geometry and volume of the developing container can also affect the separation. Narrow rectangular tanks and a moderate volume of the developing solvent provide better resolution.

Ag-TLC plates are normally developed at ambient temperature, but some improved resolution of fatty acid isomers requires a temperature of $-20^{\circ}C$. It is assumed that resolution improves because the stability of the Ag^{+} complexes increase when the temperature decreases. This might be true, but the properties of the sorbent and mobile phase also change at low temperatures. The action of all three factors is probably responsible for the better separations.

Various solvents are used to give two or three component mobile phases. Some of those most frequently used for separation of fatty acids and triacylglycerols are listed in **Table 5**. Plates are often developed more than once to improve resolution. The separation should start with the most polar phase and proceed, after drying between runs, with mobile phases of gradually decreasing polarity. In this way, highly unsaturated components are resolved first and do not move further with subsequent developments when the more saturated components are separated. Obviously, the separation will improve substantially if a continuous development can be applied. In a simple approach, which has been used for the analysis of fatty acids and triacylglycerols, the development proceeds in an open cylindrical tank where a fixed volume (4–15 mL) of the mobile phase has been added. As the mobile phase is eluted through the plate, it is permitted to evaporate from the upper edge. Resolution was very effective and excellent results have been reported including the separation of positional isomers of triacylglycerols. This open system is quite sensitive towards the laboratory environment but operates very well in skilled hands.

Detection

All detection reagents that are suitable for visualizing compounds separated on plain silica plates are, in principle, suitable for use with Ag-TLC, particularly when the percentage of silver ions in the layer is below 2%. Destructive procedures are used for location, for identification and, to some extent, for quantification purposes. The reagent can be introduced by spraying, by treatment of the plates with its vapours or by incorporating into the layer. Some of the most commonly used detecting reagents for lipids are listed in **Table 6**. Solutions of chlorosulfonic acid in acetic acid, ethanolic phosphomolibdic acid and antimony

DropBooks

Table 5 Examples of mobile phases used to separate fatty acids and triacylglycerols by Ag–TLC

Compound	*Mobile phase composition, by volume*	*Development*
Fatty acids with 0–3 double bonds	Hexane–diethyl ether, 90 : 10 or 80 : 20 Hexane–acetone, 100 : 4	One-fold development in closed tanks. One-stage development in open cylindrical tanks. *E*- and *Z*-monounsaturated fatty acids are clearly separated.
Fatty acids with 0–6 double bonds	First plate: hexane–diethyl ehter, 90 : 10 Second plate: hexane–diethyl ether, 60 : 40	Fatty acids are separated on two different plates. Species with 0–3 double bonds are separated on the first plate. Polyunsaturated species are separated on the second plate.
Triacylglycerols with 0–6 double bonds	Hexane–diethyl ether–acetic acid, 94 : 4 : 2 Benzene–ethyl acetate, 9 : 1 Benzene–diethyl ether, 85 : 15 Hexane–diethyl ether, 80 : 20 Chloroform–methanol, 96 : 4	All components are separated on a single plate by one-stage development. These mobile phases are used for both analytical and preparative separations in closed tanks.
	Hexane–acetone, different proportions	Development in open tanks with specified volume of the mobile phase, see the example in Figure 4.

Table 6 List of frequently used staining reagents for detecting lipids in Ag-TLC

Reagent	*Comments*
25–70% sulfuric acid	A destructive reagent, usually applied by spraying as solution in ethanol. Spraying must be very thorough and even, especially if the plate is considered for densitometric quantification. Spots are detected by heating the plate at temperatures of 150–200°C.
3% aqueous solution of copper acetate in phosphomolybdic acid	Advantageous in that precoated plates can be immersed in the solution, thus providing an even staining. Spots are visualized by heating.
Sulfuryl chloride	A highly volatile destructive reagent that allows convenient treatment of the plate in a closed container. This treatment provides even staining when the plate is heated. Suitable for densitometric quantification of lipids.
Rodamin 6G, 2,7-dichlorofluorescein	Nondestructive reagents used for preparative isolation of material for further examination. Plates are sprayed with diluted (>1%) solutions of the reagents in acetone. Spots are detected by viewing under UV light. The isolated material is purified from the detecting reagent by elution through a small silica gel column.

perchlorate in chloroform have been used to detect terpenes separated by Ag-TLC.

Spraying is a rapid but inconvenient and quite hazardous operation and should be avoided if and when possible and replaced by treatment with vapours. Incorporation of the charring reagents into the layer should be performed with circumspection, since it may change the nature of the resolution.

For preparative purposes, after detection, the separated zones are carefully scraped from the plate and the compounds are extracted from the sorbent with suitable polar solvents. As such material is likely to be subjected to further analysis, the extracts should be purified first. For example, in lipid analysis, excess silver ions and 2,7-dichlorofluorescein can be removed by passing the extract through small silica columns or by washing with bicarbonate, ammonia or sodium chloride solutions.

Identification

An advantage of Ag-TLC is the easy identification of the separated components with a substantial degree of certainty. A reference compound, or reference mixture of compounds, is usually applied beside the sample. The reference and the sample are developed simultaneously and this allows the migration distances (the R_F values) to be compared. Fatty acids and triacylglycerols form, for example, mixed zones with the matching reference components. In case of ambiguity, preparative Ag-TLC is applied to isolate and collect the component(s) in question for subsequent spectral analysis.

Quantification

As in all TLC techniques indirect and direct approaches for quantification have been employed. The most widely used procedure involves scraping off the detected zone and eluting the component(s) with suitable solvent. Then any of the available chromatographic or spectral techniques for quantification can be applied. Scanning densitometry can be used to measure the quantities of the separated compounds after a carefully chosen staining procedure. Staining is required since even UV-absorbing or UV-tagged compounds have either a very weak, or even no, signal in the presence of Ag^+ in the layer. Procedures have been reported for reliable densitometric quantification of fatty acids and triacylglycerols without the need for calibration graphs and correction coefficients, for example. **Figure 2** presents the densitometric profile of a triacylglycerol mixture separated by Ag-TLC.

Interactions in Argentation TLC

The rules of complex formation, presented above, have been found to be generally valid in the majority of separations performed by Ag-TLC. In many cases it is possible to predict the migration order. Interaction of Ag^+ with compounds that have more than two double bonds, however, seems to be less well understood. This primarily concerns lipids. A mixture of fatty acids, for example, may comprise components of different chain length (from 12 up to 22 carbon atoms) and of zero to six double bonds in the

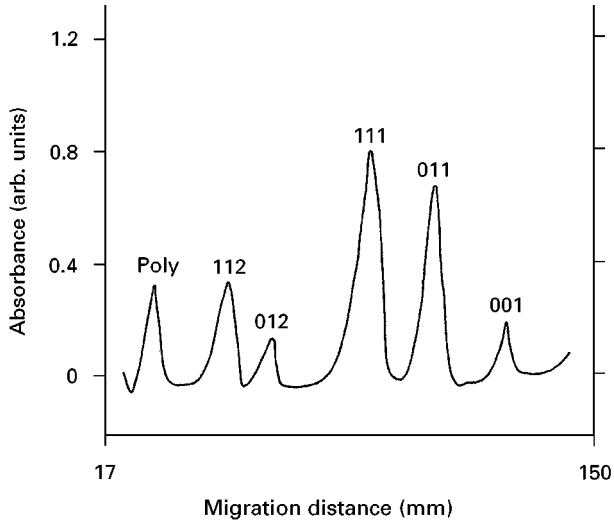

Figure 2 Densitogram of olive oil triacylglycerols separated by Ag-TLC. Conditions: laboratory-made 5 cm × 20 cm glass plate, 0.2 mm thick silica gel layer impregnated with 0.5% methanolic silver nitrate (by dipping); sample size: 20 μg; mobile phase: 6 mL light petroleum–acetone–ethyl acetate, 100 : 3 : 2 (by volume); one-stage development in an open cylindrical tank; detection: successive treatment with bromine (30 min) and sulfuryl chloride (30 min) vapours followed by carbonization by heating at 180–200°C on a temperature-controlled hot plate; scanning: Shimadzu CS-930 densitometer in zigzag mode; beam dimensions: 1.2 mm × 1.2 mm; working wavelength: 450 nm. Peak identity: Poly, minor components, containing octadecatrienoic fatty acid; the figures indicate the number of double bonds in the acyl residues but not their position in the glycerol backbone.

DropBooks

chain. Double bonds can be either *cis* (*Z*) or *trans* (*E*) or both and can have different position in the chain. Triacylglycerols and glycerophospholipids are complex mixtures of species that differ in the type and position of the acyl residues. So far, there are no theoretical or experimental studies on the mechanism of complex formation between Ag^+ and complicated unsaturated molecules. Almost nothing appears to be known about the electronic and steric effects in these molecules and their possible influence on complexation.

Attempts have been made to present the complexation of lipids in Ag-TLC in quantitative terms. Estimation has been made on the basis of the chromatographic retention of different molecular types. For example, arbitrary values of $0, 1, 2 + 2a$ and $4 + 4a$, where $a < 1$, have been proposed for the complexing power of stearic, oleic, linoleic and linolenic fatty acids (with 0, 1, 2 and 3 double bonds, respectively). Evidently, the increase in the complexing power values is greater than the increase in the number of double bonds. More accurate equations have been proposed but, in general, the values are close to those determined earlier and confirm the above conclusion. It has also been assumed that the respective values for the tri- or diacylglycerols can be expressed as a simple sum of the values for the fatty acyl residues. The assumption considers that the contribution of a fatty acid in a complex lipid molecule is not affected by the strength and properties of the silver ion complexes with neighbouring fatty acids in the same molecule. Such models are too simple and do not take account of the steric factors that may especially affect the complexation of a triacylglycerol molecule.

The role of the support material must be also taken into account. The most widely used support, silica gel, possesses appreciable polarity and absorption activity. Therefore, the retention of unsaturated compounds cannot be ascribed to the complexation reaction with Ag^+ and double bonds only, although clearly that is a major factor. The retention of an unsaturated molecule in any such system is the result of a mixed retention mechanism. For example, an unsaturated fatty acid methyl ester has been assumed to complex with the silver ions through its double bond(s) and to interact with the silanol moieties through its methyl ester group. Depending on the position of the double bond, the molecules will have different conformations. Those molecules may be held more strongly when the distance between the double bond and the ester group has a better fit with the distance between the silver ion and the silanol moiety. These two interactions have been suggested in explanation of the specific migration patterns of positionally isomeric fatty acid methyl esters. A mixed retention mechanism should be taken into account in the case of unsaturated compounds with other polar functional groups which are subjected to Ag-TLC.

A separate but related problem is the topology of silver ions on the adsorbent surface. For example, it was found that part of the silver nitrate remained in crystalline form, filling the pores of the silica gel after drying the plate to make the layer active. However, a proportion of the silver nitrate remained dissolved in the water, which is always bound to silica gel. The aqueous silver nitrate was assumed to be responsible for complex formation. Saturation of the silica layer with water before separation has even been proposed in order to obtain a pure complexation reaction. If the excess silver nitrate does indeed remain in a crystalline form and does not take part in complexation, impregnation of the layer with a highly concentrated solution of silver nitrate is of no practical value. Some of the results obtained with Ag-TLC seem to confirm this observation.

Retention and Resolution of Unsaturated Compounds in Ag-TLC: Examples

In general terms, acyclic unsaturated compounds migrate and can be resolved by Ag-TLC depending on the number, configuration and, occasionally, on the position of the double bond in the molecule, the number of the double bonds being the governing feature. The separation of fatty acids and triacylglycerols illustrates very well the resolution ability of Ag-TLC.

The migration pattern of fatty acids with zero to six double bonds is presented in **Figure 3**. For qualitative purposes it is possible to resolve fatty acids with unsaturation in the above interval on a single 5 cm × 20 cm plate using two solvent systems in sequence. Migration cannot always be predicted in the case of a mixture of fatty acids of different chain lengths. According to the general rules, when chain length increases, stability of the complex with silver ions decreases. This means that longer chain fatty acids will migrate ahead of shorter chain species of the same unsaturation (note the place of docosatetraenoic, 4a, and of octadecatetraenoic, 4b, fatty acids on Figure 3). The migration order of triacylglycerols follows the same rules, i.e. species with up to nine double bonds and acyl residue chains of 16–18 carbon atoms are ordered according to the increasing retention: 000, 001, 011, 002, 111, 012, 112, 003, 112, 013, 113, 222, 023, 123, 223, 133, 233, 333 (the figures indicate the number of double bonds in the acyl moiety but not their position in the molecule). It should be noted that of two species with an equal number of double bonds that in which all (or most) of the double bonds are concentrated into one fatty acyl moiety is held more firmly (011 and 002, for example). The separation of natural triacylglycerol mixtures depends strongly on the quantitative proportions between the components. For example, a mixture of components with relative high saturation can be resolved on a single TLC plate by the successive use of two mobile phases of decreasing polarity as illustrated in **Figure 4**. For satisfactory resolution of plant triacylglycerols with up to nine double bonds three different plates and two-stage development of each plate with mobile phases of different polarity is required. The approach provides accurate identification and densitometric quantification of the separated species.

E- and *Z*-isomers are normally easy to distinguish. An example of the separation of a fatty acid mixture is shown in **Figure 5**. Argentation TLC is may be the easiest and cheapest way to determine *trans*-monoenes in dietary fats.

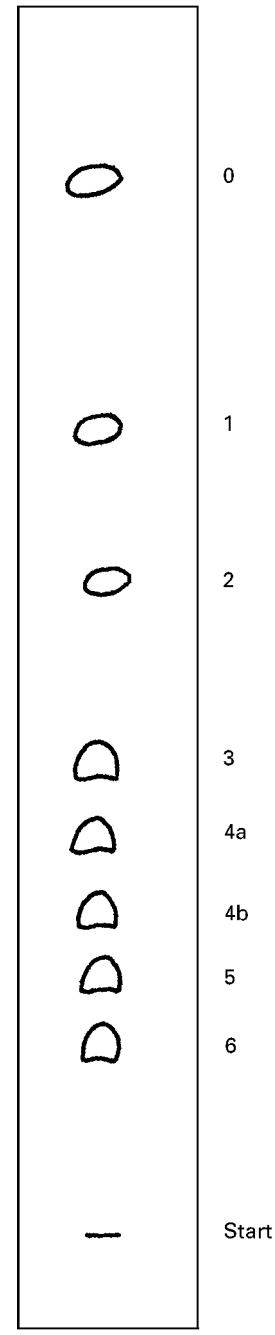

Figure 3 Migration pattern of fatty acid methyl esters with zero to six double bonds in Ag-TLC. Conditions: laboratory-made 5 cm × 20 cm glass plate, 0.2 mm thick silica gel layer impregnated with 0.5% methanolic silver nitrate (by dipping); mobile phase: 5 mL light petroleum–acetone–formic acid, 97 : 2 : 1 (by volume); one-stage development in an open cylindrical tank; detection: as in Figure 2. Spot identity: the figures indicate the number of double bonds; 4a, methyl docosatetraenoate; 4b, methyl octadecatetraenoate.

Under specified conditions Ag-TLC differentiates between mono- or diunsaturated fatty acids with different position of the double bond(s) in the carbon chain. In 1970 Gunstone and colleagues supposed

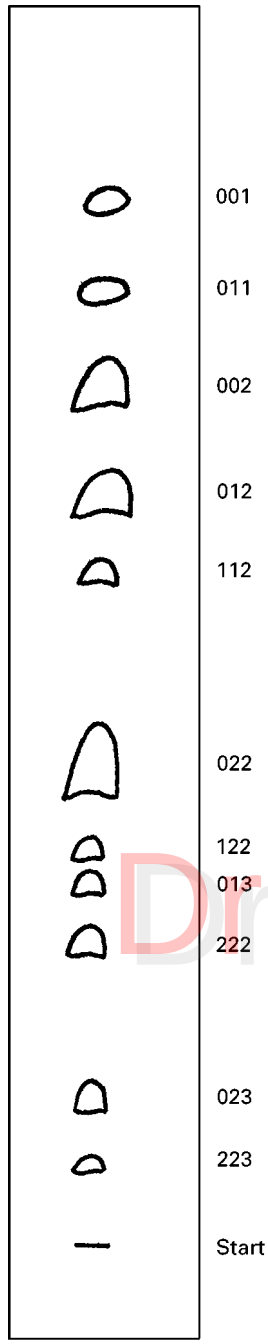

Figure 4 Migration pattern of coffee triacylglycerols. Conditions: laboratory-made 5 cm × 20 cm glass plate, 0.2 mm thick silica gel layer impregnated with 0.5% methanolic silver nitrate (by dipping); sample size 50 μg; two-stage development with 4 mL light petroleum–acetone, 100 : 4 (by volume) followed by 15 mL light petroleum–acetone, 100 : 4 (by volume); detection: as in Figure 2. Spot identity: the figures indicate the number of double bonds in the acyl residues but not their position in the glycerol backbone.

that this is due to the participation of the fatty acid molecule in additional reaction(s) with either the silver ions or with the adsorbent. Reliable resolution was achieved in isolated cases only. The approach was therefore of hardly any practical value until recently, when it was shown that separation depends strongly on the nature of the ester moiety. The effect is demonstrated in **Figure 6** and has been assigned to the participation of the ester group and the double bond in simultaneous complexation with a silver ion to give a chelate-type complex.

Of practical value is the resolution of triacylglycerols that differ by the position of the unsaturated fatty acid residue in the triacylglycerol molecule (**Figure 7**). Since the position of acyl residues is strictly specific in natural triacylglycerol mixtures, Ag-TLC provides an easy way to distinguish between natural and modified edible fats and oils and the approach is of value to the food industry.

Conclusion

Ag-TLC is a valuable qualitative and quantitative method for the separation of unsaturated compounds, lipids in particular. Ag-TLC has the advantages of rapidity, simplicity and versatility and does not require expensive instrumentation. The information obtained reflects the whole sample, thus helping the analyst to make rapid, correct and efficient judgements. It is widely used as a preliminary step in combination with other chromatographic techniques such as GC and HPLC. Sufficiently pure

DropBooks

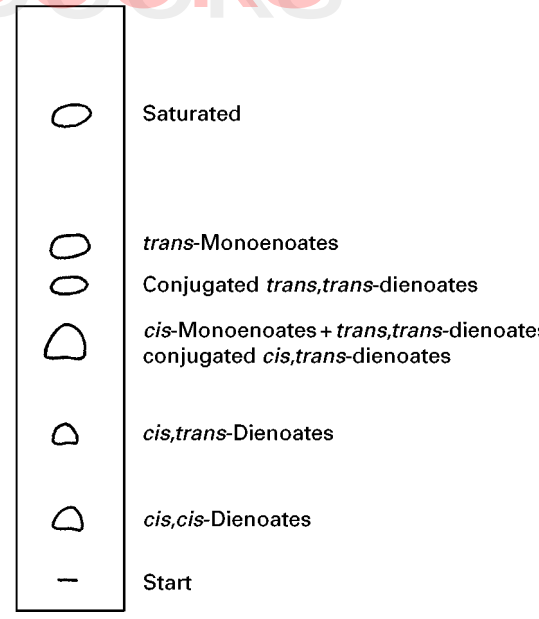

Figure 5 Migration pattern of *E*-, *Z*-fatty acid methyl esters. Conditions: laboratory-made 5 cm × 20 cm glass plate, 0.2 mm thick silica gel layer impregnated with 0.5% methanolic silver nitrate (by dipping); two-stage development with 2 mL light petroleum–acetone, 100 : 2 (by volume) followed by 3 mL light petroleum–acetone, 100 : 0.7 (by volume); detection: as in Figure 2.

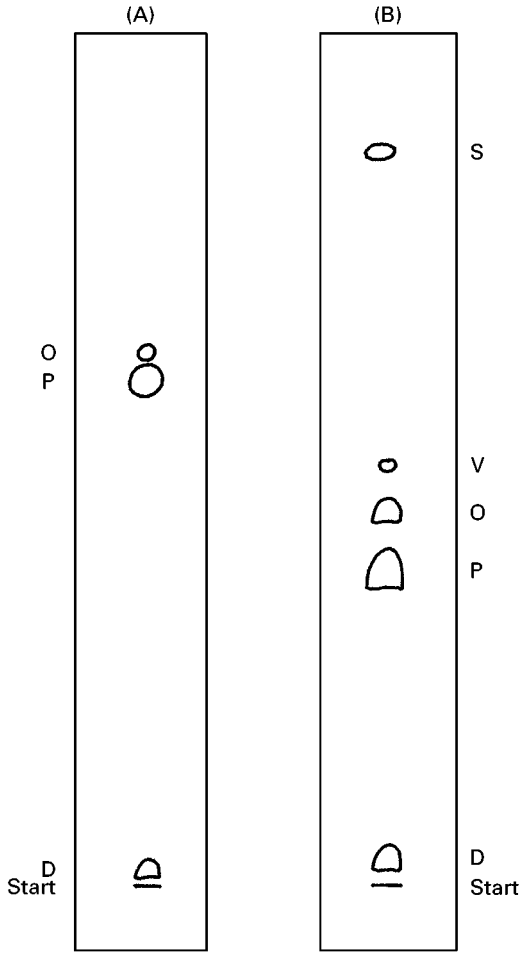

Figure 6 Migration pattern of mono-unsaturated octadecenoic fatty acids differing in the position of the double bond in the carbon chain. Sample: aniseed oil. Conditions: laboratory-made 5 cm × 20 cm glass plate, 0.2 mm thick silica gel layer; detection: as in Figure 2. (A) Separation of the fatty acid as methyl esters. The layer is impregnated with 1% methanolic silver nitrate (by dipping); development is in an open cylindrical tank with light petroleum–acetone, 100 : 5 (by volume) at -20°C. (B) Separation of the fatty acids as phenacyl esters by two-stage development in a closed cylindrical tank (no preliminary saturation of the atmosphere) with a mobile phase of chloroform–acetone, 100 : 0.25 (by volume) at ambient temperature. Note the improved resolution achieved after conversion of the fatty acids into phenacyl esters. Spot identity: S, saturated fatty acids, V, vaccenic acid, 11–18 : 1; O, oleic acid, 9–18 : 1; P, petroselinic acid, 6–18 : 1; D, linoleic acid, 9, 12–18 : 2 (position of double bond-number of carbon atoms : number of double bonds).

components can be collected for further structural elucidation by spectral methods. Combined with scanning densitometry, Ag-TLC meets all requirements of a reliable quantitative method for determination of positional and configurational fatty acid isomers and of triacylglycerols in natural samples.

The resolution power of Ag-TLC will undoubtedly increase if and when an automatically controlled system for continuous development with a sequence of different mobile phases becomes available.

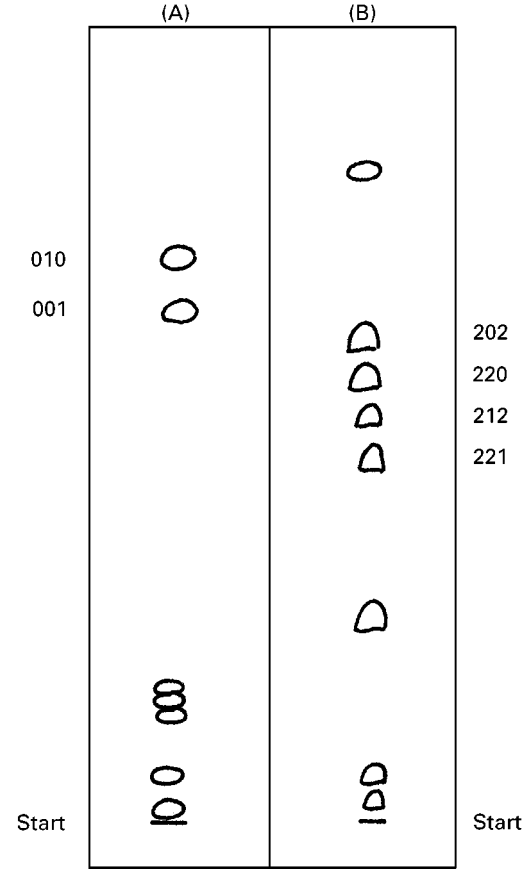

Figure 7 Migration pattern of positionally isomeric triacylglycerols by Ag-TLC. Conditions: laboratory-made 5 cm × 20 cm glass plate, 0.2 mm thick silica gel layer. (A) Sample: randomized lard; layer impregnated with 1% methanolic silver nitrate; one-stage development in an open cylindrical tank with 12 mL chloroform–methanol, 95.5 : 0.5 (by volume). (B) Sample: randomized sunflower oil; layer impregnated with 2% methanolic silver nitrate; one-stage development in an open cylindrical tank with chloroform–methanol, 97.5 : 2.5 (by volume). Spot identity: the figures indicate the number of the double bonds and the position of the acyl residue in the glycerol backbone.

See also: **II / Chromatography: Thin-Layer (Planar):** Instrumentation; Layers; Modes of Development: Conventional; Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal; Spray Reagents. **III / Impregnation Techniques: Thin-Layer (Planar) Chromatography. Lipids:** Gas Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Silver Ion:** Liquid Chromatography.

Further Reading

Ackman RG (1991) Application of thin layer chromatography to lipid separation: neutral lipids. In: Perkins EG (ed.) *Analysis of Fats, Oils and Lipoproteins*, pp. 60–82. Champaign, IL: American Oil Chemists' Society.

Cagniant D (ed.) (1992) *Complexation Chromatography*. New York: Marcel Dekker.
Christie WW (1987) *High Performance Liquid Chromatography and Lipids*. Oxford: Pergamon.
Christie WW (1982) *Lipid Analysis*, 2nd edn. Oxford: Pergamon.
De Ligny CL (1976) *Advances in Chromatography* 14: 265–304.
Fried B and Sherma J (1996) *Practical Thin-Layer Chromatography – A Multidisciplinary Approach*. Boca Raton, FL: CRC.
Fried B and Sherma J (1998) *Thin-Layer Chromatography – Techniques and Application*, 4th edn. New York: Marcel Dekker.
Gmelin Handbuch der Anorganischen Chemie (1975) *Silver*, vol. 61, TI.B5. Berlin: Springer-Verlag.
Morris LJ (1966) Separation of lipids by silver ion chromatography. *Journal of Lipid Research* 7: 717–732.
Morris LJ and Nichols BW (1972) Argentation thin layer chromatography of lipids. In: Niedewieser A (ed.) *Progress in Thin Layer Chromatography and Related Methods*, vol. 1, pp. 74–93. Ann-Arbor, MI: Ann-Arbor-Humphrey Science Publishers.
Nikolova-Damyanova B (1992) Silver ion chromatography and lipids. In: Christie WW (ed.) *Advances in Lipid Methodology – One*, pp. 181–237. Ayr: The Oily Press.

SODIUM CHLORIDE: CRYSTALLIZATION

R. M. Geertman, Akzo Nobel Chemicals Research, Arnhem, The Netherlands

Copyright © 2000 Academic Press

Introduction

Salt has been a part of human existence since time immemorial. It was used for cooking wheat and barley as early as 5000 BC. The first salt was gathered from shallow lagoons where seawater could evaporate. Later rock salt was mined, and in the Alps, for instance, rock salt is known to have been mined as early as 1400 BC. Because of its importance to human life, salt has had an influence on economy, history and culture. Many sayings and words are derived from the use of salt: e.g. 'to be worth one's salt' is a compliment, the Bible speaks of 'the salt of the earth' and soldier is derived from the Latin '*sal dare*' which means to give salt.

Indeed, in ancient times salt had a much greater value than it has nowadays. It was traded weight by weight with gold, and the salt trade was very profitable. The Hanseatic League started by trading in salt. Taxes on salt were very common, and in that sense salt has played a role in many important historic events. The French Revolution was partly in protest against salt taxes. Gandhi's campaign of civil disobedience, which eventually led to the independence of India, started when he evaded the British salt monopoly by producing salt himself.

Uses of Salt

Before the industrial revolution and the discovery of the electrolysis process, the uses of salt were limited. The main uses were for the cooking, preserving and pickling of food and the tanning of hides. These uses are still very important, as salt is essential for the human body. With the development of chemical processes the uses of salt have diversified enormously. Apart from uses in the food industry, salt is, for instance, used in dyeing, paper production, highway de-icing, oil well drilling and the production of soda ash. Electrolysis of salt is the major source of chlorine and sodium hydroxide for the chemical industry. Chlorine is essential for the production of a number of plastics, insecticides and pharmaceutical compounds. Either directly, or in the form of derivatives, salt finds application in more than 14 000 ways. This multitude of applications can be divided into three major categories: chemical uses, highway de-icing, and food-related uses. In the industrialized nations the chemical industry accounts for approximately 50% of the salt consumption, and highway de-icing for about 30% while food applications make up the remainder. In developing countries most of the salt produced is used in food.

Production of Salt

As salt is the most abundant nonmetallic mineral, most countries have the ability to produce salt. It is so abundant that it is hard to estimate salt reserves. In the United States alone, reserves are estimated at 55 trillion tonnes. In 1996, 192 million tonnes of salt were produced, approximately 55% in the industrialized nations and about 45% in developing countries. Details are given **Table 1**.

DropBooks